Docket No.: C1233.70001US01
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Alejandro Balazs et al.
Serial No.:	10/577,177
Confirmation No.:	4085
Filed:	February 5, 2007
For:	METHODS FOR PURIFYING HEMATOPOIETIC STEM CELLS
Examiner:	M. K. Sgagias
Art Unit:	1632

DECLARATION OF ALEJANDRO BALAZS UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

1. I, Alejandro Balazs, am a scientist at California Institute of Technology and have extensive training and experience in the fields of hemapoietic stem cell biology, flow cytometry and immunology. I received my Ph.D. in Genetics in 2006 from Harvard. A copy of my Curriculum Vitae is attached as Exhibit 1.

2. I am an inventor named on and have read the above-identified patent application. I have also read the pending claims, the Office action mailed June 24, 2010 and the Ghannadan et al. reference cited in the action.

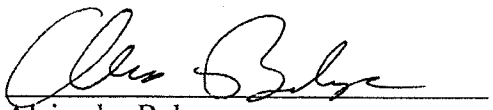
3. In the Office action, the Examiner asserts that the Ghannadan et al. reference anticipates the instant invention. The Examiner's conclusion is based on an incorrect characterization of Ghannadan et al. The assertion that 'Ghannadan et al. disclose a) contacting a biological sample of HMC-1 cell line with the mAb for endothelial cell C protein (CD201) and b) separating cells that bind to the mAb by FACS analysis thereby producing a substantially pure population of EPCR+ hematopoietic stem cells' (pages 2-3 of the Office Action) is incorrect. The HMC-1 cell line, which is a mast cell line, does not contain any hematopoietic stem cells and, therefore, it is not possible to obtain hematopoietic stem cells using HMC-1 and an endothelial cell C protein

antibody. Mast cells are highly specialized cells found in tissues throughout the body and play a role in allergy and anaphylaxis. Unlike hematopoietic stem cells, mast cells do not have the capacity to reconstitute the entire hematopoietic system. The mast cell line cannot produce nor does it contain any hematopoietic stem cells. Therefore, it would not have been possible to obtain hematopoietic stem cells using HMC-1 and an endothelial cell C protein antibody.

4. Studies aimed at understanding the basic biology of hematopoietic stem cells are undermined by an inability to obtain purified hematopoietic stem cells. The instant application identified, for the first time, Endothelial Protein C Receptor (EPCR) as a novel marker for hematopoietic stem cells. The use of a single EPCR marker to isolate a substantially pure population of hematopoietic stem cells is one of the key innovations described in the referenced application.

5. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and, further, that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above application and any patent or application related thereto.

Date: Sept 22nd, 2010

By: 
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Education

1995-1999:

BS – Microbiology and Molecular Genetics
Departmental Honors, College Honors, Cum Laude
University of California at Los Angeles, Los Angeles CA

1999-2006:

PhD – Genetics
Laboratory of Richard Mulligan
Harvard Medical School, Boston MA

Honors and Awards

- The Foundation for AIDS Research (Amfar) Postdoctoral Fellowship (2010-Present)
- University of California Chancellor Scholarship
- UCLA Alumni Association Scholarship 1995-1996
- Vice Provost Recognition Award, UCLA
- Elma Gonzalez Dean's Prize for Undergraduate Research, UCLA
- MARC Scholar, UCLA
- Cum Laude, UCLA
- College Honors, College of Letters and Science - UCLA
- Departmental Honors, Department of Microbiology and Molecular Genetics - UCLA
- National Science Foundation Graduate Fellowship (Honorable Mention)

Research Experience

October 2007 – Present: Engineering Immunity to HIV utilizing Viral Vectors
California Institute of Technology, Department of Biology
Postdoctoral research

Postdoctoral fellow engineering a non-hematopoietic immune system using adeno-associated viral vectors as a potential vaccine approach against HIV. Created an AAV vector system to express broadly neutralizing anti-HIV antibodies in vivo and developed a humanized mouse model capable of sustaining HIV infection in which to test potential vaccine candidates.

Principal Investigator: David Baltimore, Professor of Biology – California Institute of Technology

September 1999 – June 2006: Characterization of Hematopoietic Stem Cells and Viral Vector Development
Harvard Medical School, Department of Genetics
Graduate level research towards the fulfillment of the requirements of a PhD

PhD student using microarray analysis of hematopoietic stem cells to characterize the expression program of HSC. Identified CD201 as a highly specific marker of HSC and demonstrated its use in a simple and effective stem cell purification method. Designed and constructed a next-generation accessory protein independent lentivirus vector system to simplify genetic manipulation of target cells including HSC.

Principal Investigator: Richard Mulligan, Professor of Genetics – Harvard Medical School

January 1998 – June 1999: Anti-HIV Immunotherapeutics
UCLA, Department of Microbiology and Immunology
Undergraduate Research performed towards completion of departmental honors thesis, MARC fellowship and summer research program

Undergraduate honors thesis research student using standard molecular biological techniques to design, construct, express, purify and test novel chemokine-antibody fusion proteins for use as potential HIV immunotherapeutics.

September 1997 – January 1998: Chimeric Immunoglobulins
UCLA, Department of Microbiology and Immunology
Undergraduate research performed during student research program

Undergraduate summer research student working towards the creation of chimeric immunoglobulins that combined favorable properties of IgA and IgG for use as new protein immunotherapeutics.

Principal Investigator: Sherie Morrison, Chair of Microbiology & Immunology - UCLA

June 1996/7 – September 1996/7: Atomic Force Microscopy of Biological Tissues
LLNL, Materials Sciences Division
Lawrence Livermore National Laboratories summer research program

Undergraduate summer student studying the mechanical properties of biological tissues using atomic force microscopy and nanoindentation. Performed imaging and indentation experiments and created software to automate data analysis and significantly increase throughput.

Principal Investigator: Mehdi Balooch, Professor of Materials Science – UC Berkeley

Publications

Balooch M, Wu-Magidi IC, **Balazs A**, Lundkvist AS, Marshall SJ, Marshall GW, Siekhaus WJ, Kinney JH.
Viscoelastic properties of demineralized human dentin measured in water with atomic force microscope (AFM)-based indentation. J Biomed Mater Res. 1998 Jun 15;40(4):539-44.

Michurina T, Krasnov P, **Balazs A**, Nakaya N, Vasilieva T, Kuzin B, Khrushchov N, Mulligan RC, Enikolopov G.
Nitric oxide is a regulator of hematopoietic stem cell activity. Mol Ther. 2004 Aug;10(2):241-8.

Balazs AB, Fabian AJ, Esmon CT, Mulligan RC.
Endothelial protein C receptor (CD201) explicitly identifies hematopoietic stem cells in murine bone marrow. Blood. 2006 Mar 15;107(6):2317-21. Epub 2005 Nov 22.

Austin KM, Gupta ML, Coats SA, Tulpule A, Mostoslavsky G, **Balazs AB**, Mulligan RC, Daley G, Pellman D, Shimamura A
Mitotic spindle destabilization and genomic instability in Shwachman-Diamond syndrome.J Clin Invest, 2008 Apr;118(4):1511-8

O'Connell RM, Chaudhuri AA, Rao DS, Gibson WS, **Balazs AB**, Baltimore D
MicroRNAs enriched in hematopoietic stem cells differentially regulate long-term hematopoietic output. Proc Natl Acad Sci, 2010 Aug 10;107(32):14235-40

Balazs AB, O'Connell RM, Rao DS, Kivork C, Yang L, Baltimore D
Lentiviral Vector Delivery of Human Interleukin-7 (hIL-7) to Human Immune System (HIS) Mice Expands T Lymphocyte Populations. PLoS One, 2010 Aug 6;5(8). pii: e12009

Balazs AB, Mulligan RC
Development of improved lentiviral vectors and methods of vector production for pre-clinical and clinical studies. Manuscript in Preparation.

Presentations

September 2010: Web-Presentation
A.B. Balazs, O'Connell RM, Rao DS, Kivork C, Yang L, Baltimore D: Lentiviral Vector Delivery of Human Interleukin-7 (hIL-7) to Human Immune System (HIS) Mice Expands T Lymphocyte Populations.
NIH Webinar, HIS-Humanized Immune Systems of Mice

August 2010: Oral Presentation
A.B. Balazs, J. Tsai, D. Baltimore: Isolation of Unknown Rearranged T-Cell Receptors from Single Cells via Whole Genome Amplification
International Congress of Immunology 2010, Kobe Japan

June 2003: Poster Presentation

A.B. Balazs, A.J. Fabian, R.C. Mulligan: A Novel Marker for Hematopoietic Stem Cells
International Society of Stem Cell Research , Washington DC

March 2003: Poster Presentation
A.B. Balazs, A.J. Fabian, R.C. Mulligan: A Novel Marker for Hematopoietic Stem Cells
Hematopoiesis Keystone Symposia , Steamboat Springs CO

September 1998: Poster Presentation
A.B. Balazs, S.L. Morrison, K.C. Chintalacharuvu: Generation of SDF-Ab Fusion Proteins
CARE Scholars Summer Research Program, Los Angeles CA

Courses/Lectures

April 29th, 2010: Introduction to Gene Therapy
Bio 115 - Virology, California Institute of Technology

References

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